

43. The method of claims 16 or 17, wherein the physiologically acceptable gas is selected from the group consisting of  $\text{CF}_4$ ,  $\text{C}_2\text{F}_6$ ,  $\text{C}_4\text{F}_8$ , or  $\text{C}_4\text{F}_{10}$ .

44. The method of claim 16, wherein the physiologically acceptable gas is  $\text{CF}_4$ .

45. The method of claim 16, wherein the physiologically acceptable gas is  $\text{C}_2\text{F}_6$ .

46. The method of claim 16, wherein the physiologically acceptable gas is  $\text{C}_4\text{F}_8$ .

47. The method of claim 16, wherein the physiologically acceptable gas is  $\text{C}_4\text{F}_{10}$ .

48. The method of claim 16, wherein the physiologically acceptable gas is  $\text{SF}_6$ .

### **REMARKS**

Claims 1-3, 6, 7, 13-22, 25, 26, and 30-48 are pending in this application. Applicants note that claims 6 and 25 remain pending. Despite being inadvertently and incorrectly included in the cancellation instruction in the beginning of the previous Amendment filed December 7, 2000, claims 6 and 25 were amended and reprinted in the previous Amendment and also discussed throughout the Remarks section of the previous Amendment as well. Thus, the Examiner is respectfully requested to reinstate and reconsider claims 6 and 25 with the other pending claims in light of the remarks below.

Claims 16, 17, 19, 20, 33, and 34 have been amended, not for reasons related to patentability, but to better define the Applicants' microballoon invention by inserting the language "consisting of" as previously suggested by the Examiner.

In a new ground of rejection, Applicants' pending claims have been rejected under 35 U.S.C. § 103 over Cerny, Ryan, or Hilmann, in view of Glajch, Quay, Tickner and the DuPont bulletin. Applicants respectfully traverse.

Applicants believe that these claims are patentable for the same reasons that the original claims were patentable as explained in Applicants' March 29, 2000 Amendment And Response To Office Action and December 7, 2000 Preliminary Amendment. They are supported in the specification as shown in Applicants' Preliminary Amendment filed July 15, 1998, and Applicants are not aware of any prior art that has all of the elements of the claims or which in proper combination with other prior art would provide all of the elements of the claims.

Applicants understand that the Examiner is aware that U.S. Patent No. 5,413,774, the parent patent from which this '963 reissue application was filed, is involved in Interference No. 103,880. Applicants further understand that the Examiner is also aware of other pending, resolved or requested interferences relating to the general subject matter of this application and, thus, Applicants encourage the Examiner to consider them as well.

I. Applicants' Claimed Stabilized Microbubbles

Applicants' claimed invention relating to this subject matter is directed to methods of making a contrast agent consisting of gas filled stabilized microbubbles in which a bubble of freon gas is stabilized by a layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface.

Applicants discovered that contrast agents containing freon gas gave superior imaging results compared to other known gases such as air or nitrogen because the contrast agents remained longer in the bloodstream than known contrast agents made with other gases. See, e.g., the comparison in Examples 4-6. Applicants also discovered that contrast agents made with the claimed phospholipids were superior to other known substances or polymers used in the art such as the rigid aldehydes or saccharides because the claimed phospholipids would form a monolayer around the gas which was more resilient and better at adjusting to sudden pressure variations in the bloodstream than the known rigid polymer microparticles. This unique combination, which

is not disclosed in the prior art of record, of a freon gas surrounded by the Applicants' claimed phospholipids, gives Applicants' claimed microbubbles superior contrast agent properties compared to other contrast agents known in the art.

None of the cited references, viz, Cerny, Ryan, or Hilmann, in view of Glajch, Quay, Tickner and the DuPont bulletin, may be combined to disclose the Applicants' claimed stabilized microbubble invention. Specifically, there is no proper combination of these references which will disclose or suggest anywhere the limitation wherein a fluorinated gas is "bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface."

Applicants' stabilized microbubbles were first disclosed in EP 90810262.7, filed April 2, 1990. However, since Applicants are not required to rely on that date to overcome the cited references, the issue of the effective filing date (determined by Examiner to be January 24, 1992) is moot.

## II. Applicants' Claimed Microballoons

Applicants' claimed invention relating to this subject matter is directed methods of making a contrast agent consisting of gas filled microballoons in which a bubble of freon gas is surrounded by an organic polymer envelope which is formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids.

As already discussed above, Applicants discovered that contrast agents containing freon gas gave superior imaging results compared to other known gases such as air or nitrogen because the contrast agents remained longer in the bloodstream than known contrast agents made with other gases. See, e.g., the comparison in Example 1. Applicants also discovered that contrast agents made with the claimed organic polymer were superior to other known polymers used in

the art such as the rigid aldehydes or saccharides because the claimed organic polymer would form an elastic shell around the gas which was more resilient and better at adjusting to sudden pressure variations in the bloodstream than the known rigid polymer microparticles. This unique combination, which is not disclosed in the prior art of record, of a freon gas surrounded by the Applicants' claimed organic polymer envelope, gives Applicants' claimed microballoons superior contrast agent properties compared to other polymer based contrast agents known in the art.

None of the cited references, viz, Cerny, Ryan, or Hilmann, in view of Glajch, Quay, Tickner and the DuPont bulletin, may be combined to disclose the Applicants' claimed microballoon invention. Specifically, there is no proper combination of these references which will disclose or suggest anywhere the limitation wherein a fluorinated gas is "bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids."

Applicants' microballoons were first disclosed in EP 90810367.4, filed May 18, 1990. However, since Applicants are not required to rely on that date to overcome the cited references, the issue of the effective filing date (determined by Examiner to be January 24, 1992) is moot.

### III. Applicants' Claims Are Patentable Over The Cited References

Applicants' pending claims have been rejected under 35 U.S.C. § 103 as allegedly being obvious over Cerny, Ryan, or Hilmann, in view of Glajch, Quay, Tickner and the DuPont bulletin. Specifically, the Examiner stated that it would have been obvious obtain the Applicants' claimed inventions by modifying the invention of Cerny, Ryan, and/or Hilmann to

substitute the gases therein with the gases taught by Glajch, Quay, and Tickner. Office Action, pp. 4-5. Applicants respectfully traverse.

Applicants respectfully submit that their claims are patentable and nonobvious over the cited references because there is no motivation to combine them, and even if there was such motivation (Applicants submit there is not), the combination of these references fail to teach or suggest each of the limitations in the Applicants' claims. A brief review of the cited references is provided below:

A. The Cited References

1. Cerny (U.S. Patent No. 4,957,656)

Cerny teaches a continuous sonication method for preparing albumin "microspheres" filled with air. Nitrogen, oxygen, and carbon dioxide may also be used. Cerny states that it solved the prior art manufacturing problem with respect to albumin "microspheres" by disclosing a method which produces "microspheres" on a continuous high production basis. Cerny further states that its "microspheres" are small, stable, and adequate for its uses. Col. 3, lines 36-40. Cerny does not suggest that its method or "microspheres" have any problems that need to be solved or improved. Furthermore, none of the other contrast agents cited by the Examiner may be produced using Cerny's methods. Thus, Cerny teaches away from combining its teachings with any of the other references cited by the Examiner.

2. Ryan (U.S. Patent No. 4,900,540)

Ryan discloses a liposome with an aqueous interior which contains gas or gas precursor. Col. 2, lines 15-17, 51-52. Thus, the interior of the Ryan liposome is a mixture of water and gas. Hence, Ryan does not disclose gas filled microbubbles or microballoons. The Examiner did not appreciate this fact in the Office Action (p. 4).

Furthermore, Ryan teaches that its liposomes are adequate for its uses and does not suggest any problems that need to be solved or any improvements that need to be made. Col. 2, lines 28-30. In fact, Ryan suggests that liposomes provide the most practical and effective encapsulation for aqueous materials (col. 1, lines 27-29), thereby distinguishing and teaching away from combining with other types of contrast agents such as those disclosed in the art cited by the Examiner.

3. Hilmann (U.S. Patent No. 4,466,442)

Hilmann discloses a solution containing a selected amount of tenside, viscosity raising compound and gas. The Hilmann disclosure states that its "microbubbles" achieve significantly superior imaging because of the presence of both the tenside and a viscosity raising compound and further distinguishes its invention from all other contrast agents such as Tickner. Col. 1, lines 51-63; col. 2, lines 30-39. By confirming the alleged superiority of its invention and claiming that it has solved the problems of the prior art, Hilmann in effect teaches away from any combination with the prior art cited by the Examiner.

Furthermore, contrary to the Examiner's statement at page 4 of the Office Action, Hilmann does not teach the use of phospholipids as envelope forming materials. In fact, Dr. Schneider, who is an inventor in this application, submitted a Declaration dated March 24, 2000 (Exhibit E to March 29, 2000 Amendment, a copy of which is attached hereto) which proves that Hilmann does not disclose the Applicants' claimed stabilized microbubbles. Schneider Declaration ¶¶ 6-17. Rather, Dr. Schneider's experiment established that Hilmann's microbubbles are free gas microbubbles which do not have any surfactant boundary surrounding the gas.

4. Glajch (U.S. Patent No. 5,147,631)

Glajch discloses microparticles made of inorganic material. These microparticles are porous and may be crystalline. The Examiner relies on Glajch for disclosing the use of CF<sub>4</sub> and C<sub>2</sub>F<sub>6</sub> gases. Office Action, pp. 4-5.

However, Glajch does not suggest anywhere that its gases can be used, or would even be useful with other contrast agents. Glajch does not even indicate anywhere that the type of gas used affects the imaging performance of the contrast agent. Rather, Glajch states that the key to a viable contrast agent is good mechanical stability and rigidity (col. 4, lines 50-54), and that the inorganic porous particles provide the contrast for ultrasound imaging. Col. 3, lines 3-4. Glajch further teaches its belief that the most desirable contrast effects are obtained by changing the shape, size or porosity of the particle. Col. 4, lines 56-59.

In fact, Glajch specifically distinguishes its invention from the albumin microspheres of Feinstein (similar to Cerny), from the saccharide particles of Tickner, as well as from the “microparticles” of Hilmann. Col. 1, lines 39-54; col. 1, line 67 - col. 2, line 8. Thus, by teaching that its inorganic particles are superior to other types of contrast agents, and by suggesting that it has solved all the problems with the mechanical stability and rigidity of the prior art, Glajch teaches away from any combination with the prior art cited by the Examiner.

5. Quay (U.S. Patent No. 5,393,524)

Quay discloses free gas microbubbles which do not have any type of envelope surrounding the gas. The Examiner relies on Quay for disclosing SF<sub>6</sub> and C<sub>4</sub>F<sub>8</sub> gases. Office Action, pp. 4-5.

However, contrary to the Examiner’s statement, Quay at col. 7-8 specifically distinguishes his invention from and criticizes the prior art inventions which may contain shell type materials such as liposomes and albumin “microbubbles”. Thus, the Examiner relies on

combinations that Quay explicitly rejects as inferior. Furthermore, Quay also states that its free gas microbubbles have “novel and superior” properties compared to other types of contrast agents. E.g., col. 1, line 18; col. 2, lines 17-21. By clearly distinguishing its invention from other contrast agents such as liposomes or albumin “microbubbles”, teaching that its free gas microbubbles are superior, and claiming that it has solved all of the problems of prior free gas microbubbles, Quay thus teaches away from combining with any of the references cited by the Examiner.

6. Tickner (U.S. Patent No. 4,265,251)

Tickner discloses the use of saccharide microparticle precursors which dissolve in the bloodstream to release free gas microbubbles. These saccharide microparticles are porous, crystalline, rigid, and preferably ground. The Examiner relies on Tickner for teaching use of freon gas. Office Action, pp. 4-5. The Examiner also states (p. 4) that Tickner discloses that the “microvesicles may be any microbubbles useful for ultrasound, see column 3.” Applicants find no such teaching in the reference. Instead, Tickner is limited to its saccharide composition.

Furthermore, there is nothing in Tickner which suggests the desirability of using its gas with any other contrast agents. Rather, Tickner states that its method is advantageous and solves the problems of the prior art by providing a solid precursor which dissolves to release free gas microbubbles. Col. 2, lines 23-36. Tickner does not suggest any problems with its invention that need to be solved or any improvements that need to be made. Thus, Tickner teaches away from any combination with the prior art cited by the Examiner.

7. DuPont Technical Bulletin

The DuPont Bulletin discloses a list of gases which are freons. To the extent the Examiner relies on DuPont to verify anything other than that the term “freon” includes the gases



in the Applicants' claims (Office Action, pp. 4-5), such reliance would be improper because there is no indication in DuPont whatsoever that these gases may be used as contrast agents.

B. There Is No Motivation To Combine

The references relied upon by the Examiner fail to provide the necessary incentive or motivation to combine them in an attempt to create the Applicants' claimed invention. There is nothing in any of the references to suggest the desirability of the combination or modification in the manner indicated by the Examiner. Thus, the combination of references proposed by the Examiner is improper and Applicants respectfully request that this rejection be withdrawn.

1. There Is No Suggestion In The Cited References To Combine

It is well-established that before a conclusion of obviousness may be made based on a combination of references, there must have been a reason, suggestion, or motivation to lead one of ordinary skill in the art to combine those references. *In re Dembiczak*, 50 U.S.P.Q.2d 1614, 1617-18 (Fed.Cir. 1999)(“Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references.”)

Merely asserting that it would have been within the skill of the art to substitute one type of gas for another in the contrast agent of the primary reference is not enough. *In re Fine*, 5 U.S.P.Q.2d 1596 (Fed.Cir. 1988)(Holding that there was no support for the Examiner's mere assertion that it would have been obvious to substitute one type of detector for another in the system of the primary reference); *In re Jones*, 21 U.S.P.Q.2d 1941 (Fed.Cir. 1992)(Holding that there was no suggestion to combine a primary herbicide reference with secondary references directed to shampoo additives or byproducts of mopholines to arrive at the claimed invention.); MPEP § 2143.01.

There is nothing in any of the cited references to suggest the desirability of the combination or modification in the manner indicated by the Examiner. In fact, each of the references Cerny, Ryan, Hilmann, Glajch, Quay and Tickner teach against the modification of their invention or the combining of parts of their invention with other references because they each teach their belief that their agents are superior to all others, with no problems or improvements needed or even suggested. It is especially significant that despite the presence of the DuPont reference, none of these contrast agent references chose to incorporate or adopt the DuPont teaching regarding gases. The Examiner's proposed combination thus would not have been made by one of ordinary skill in the art. Moreover, there are no road signs or blaze marks in the references that would lead one to ignore the bulk of their teachings and recommendations and be led to anything like Applicants' specific gases, specific stabilized microbubbles and specific polymer microballoons.

2.     The Mere Fact That References Can Be  
Modified Or Combined Is Not Enough

Further, as stated by the Court in *In re Fritch*, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992)(emphasis added):

The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification.

Thus, the mere fact that references can be combined or modified (Applicants believe they cannot be) does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 16 U.S.P.Q.2d 1430 (Fed.Cir. 1990); MPEP § 2143.01. Hence, the Examiner's attempt to combine the cited references alone without any suggestion in the references of the desirability of the modification is improper and should be withdrawn.

3.     The Modification Cannot Change  
The Principle Of Operation Of A Reference

The proposed modification cannot change the principle of operation of a reference. *In re Ratti*, 123 U.S.P.Q. 349 (C.C.P.A. 1959); MPEP § 2143.01. However, the Examiner's proposed modification would effectively change the principle of operation of each of the references. For example, Quay ("free gas microbubbles") cannot be combined with Cerny (albumin "microspheres") or Ryan (liposomes) because Quay explicitly teaches the benefits of having no surrounding materials while Cerny and Ryan both rely on the type of surrounding material for stability. Thus, the Examiner's proposed modification is improper and this rejection should be withdrawn.

4.     There Is No Reasonable  
Expectation Of Success

There also must be a reasonable expectation of success from the prior art in combining the references. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438, 1442 (Fed.Cir. 1991). This motivation to combine and the reasonable expectation of success both must be found in the prior art and not the Applicants' disclosure. *In re Vaeck*, 20 U.S.P.Q.2d at 1442. Using the Applicant's own disclosure in an obviousness analysis is considered improper and prohibited by case law. *Grain Processing Corp. v. American Maize-Products Co.*, 840 F.2d 902, 907, 5 USPQ2d 1788, 1792 (Fed. Cir. 1988)("Care must be taken to avoid hindsight reconstruction by using 'the patent in suit as a guide through the maze of prior art references, combining the right references in the right way so as to achieve the result of the claims in suit.'"); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988)("One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.")

Since each of the cited references are directed to different types of ultrasound contrast agents, there is thus no reasonable expectation of success that the agents taught by Cerny (albumin "microspheres"), Ryan (liposomes with an aqueous interior which contains gas), or Hilmann (free gas microbubbles with viscosity enhancers) will work with the gases disclosed in Glajch (inorganic microparticles), Quay (free gas microbubbles), Tickner (free gas microbubbles in saccharide microparticles) and DuPont (not for ultrasound contrast agents) as suggested by the Examiner. This conclusion is supported by the assertion by each reference of its respective superiority over the other types of contrast agents and distinction from them.

Without any reasonable expectation of success, it is improper to combine the references cited by the Examiner and withdrawal of this rejection is respectfully requested.

C.     The Cited References Do Not  
Render Applicants' Claims Obvious

Even if there exist some motivation to combine, which Applicants assert there is not, all of the claim limitations must still be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974); In re Wilson, 424 F.2d 1382, 1385, 165 U.S.P.Q. 474, 496 (CCPA 1970). MPEP § 2143.03.

Contrary to the Examiner's statement, attempting to properly modify the invention of Cerny, Ryan, or Hilmann to substitute the gases therein with the gases of Glajch, Quay, Tickner or the DuPont bulletin would still fail to teach or suggest each of the limitations in the Applicants' claims.

1.     Applicants' Claimed Stabilized Microbubbles

Cerny, Hilmann, Cerny, Glajch, Quay, Tickner and DuPont do not in any proper combination, teach, suggest, or disclose the method of making the Applicants' stabilized microbubbles wherein a fluorinated gas or gas mixture is bounded by a stabilizing layer of one or

more film forming phospholipids in lamellar or laminar form at the gas/liquid interface. The method of making Applicants' stabilized microbubbles are claimed in claims 1-3, 6, 7, 13-15, 18, 21, 25, 26, 32, 35, 37, 38-42.

Additionally, Cerny, Hillmann, Cerny, Glajch, Quay, Tickner and DuPont do not in combination, teach, suggest, or disclose forming the Applicants' stabilized microbubbles by the gas substitution method of claims 2, 3, 18, and 21.

2. Applicants' Claimed Microballoons

Cerny, Hilmann, Cerny, Glajch, Quay, Tickner and DuPont also do not in any proper combination, teach, suggest, or disclose the method of making Applicants' microballoons wherein a fluorinated gas or gas mixture is bounded by an organic polymer envelope at the gas/liquid interface, said envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin (see Example 1 in the specification), reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids. The method of making Applicants' microballoons are claimed in claims 16, 17, 19, 20, 22, 30, 31, 33, 34, 36, 43-48.

Additionally, Cerny, Hilmann, Cerny, Glajch, Quay, Tickner and DuPont do not in combination, teach, suggest, or disclose forming the method of forming Applicants' microballoons by the gas substitution method of claims 19, 20, and 22.

3. Applicants Have Proven  
Unexpected Results

In fact, Applicants were the first inventors to discover that the combination of fluorinated gases with either a stabilizing layer of phospholipids in lamellar or laminar form, or an organic polymer envelope made from polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, or esters of polyglutamic and polyaspartic acids, gave greater than expected stability and contrast duration.

*Prima facie* proof of the superiority and nonobviousness of the Applicants' stabilized microbubble and microballoon invention are explicitly disclosed in the examples of the specification. See Examples 1, 2, 4, 5, 6. Applicants have even proved that their claimed microballoons have critical pressures more than twice as great and have contrast durations more than five times longer than the closest cited prior art filled albumin microballoons (Cerny). Applicants have also submitted extra experimental evidence proving that their claimed stabilized microbubbles are far superior than those disclosed by the respective cited closest prior art Hilmann. Schneider Declaration, ¶¶ 6-17.

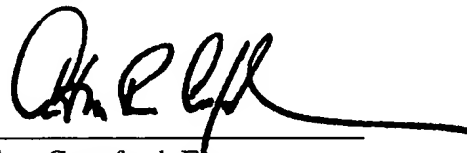
With such indisputable proof of unexpected and superior results, Applicants' claimed invention are clearly nonobvious with respect to the cited references and the Examiner is respectfully requested to withdraw this rejection under 35 U.S.C. § 103. MPEP § 716.02(a).

bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microballoons in the presence of the gas mixture comprising a physiologically acceptable gas selected from the group consisting of SF<sub>6</sub>, CF<sub>4</sub>, CBrF<sub>3</sub>, C<sub>4</sub>F<sub>8</sub>, CClF<sub>3</sub>, C<sub>2</sub>F<sub>6</sub>, C<sub>2</sub>ClF<sub>5</sub>, CBrClF<sub>2</sub>, C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub> and C<sub>4</sub>F<sub>10</sub>, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference  $\Delta P$  between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25 Torr.

**COMMENT**

See the concurrently filed Amendment for a discussion of the above claims.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Arthur Crawford', with a long horizontal line extending to the right.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In The Reissue Of	)	Examiner: M. Hartley
	)	
U.S. Pat. No. 5,413,774	)	Art Unit: 1616
	)	
Reissue Serial No. 09/115,963	)	
	)	
Filed: July 15, 1998	)	
	)	
For: Long-Lasting Aqueous Dispersions	)	
Or Suspensions Of Pressure-	)	
Resistant Gas-filled Microvesicles	)	
And Methods For The Preparation	)	
Thereof.	)	
<hr/>		October 25, 2001

Box Reissue  
Assistant Commissioner for Patents  
Washington, D.C. 20231

**37 C.F.R. § 1.121 (c)(1)(ii)**  
**MARKED UP VERSION OF THE CLAIMS**

Pursuant to 37 C.F.R. § 1.121 (c)(1)(ii), Applicants hereby submit a marked up version of the amended claims to show the changes made relative to the previous version of those claims.

**IN THE CLAIMS**

16. (Twice amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons [filled with] consisting of a physiologically acceptable gas [wherein the gas is] bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming



the microvesicles in the presence of said physiologically acceptable gas selected from the group consisting of SF<sub>6</sub>, CF<sub>4</sub>, CBrF<sub>3</sub>, C<sub>4</sub>F<sub>8</sub>, CClF<sub>3</sub>, C<sub>2</sub>F<sub>6</sub>, C<sub>2</sub>ClF<sub>5</sub>, CBrClF<sub>2</sub>, C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub> and C<sub>4</sub>F<sub>10</sub>, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

17. (Twice amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons [filled with] consisting of a gas mixture [wherein the gas mixture is] bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microvesicles in the presence of the gas mixture comprising a physiologically acceptable gas, selected from the group consisting of SF<sub>6</sub>, CF<sub>4</sub>, CBrF<sub>3</sub>, C<sub>4</sub>F<sub>8</sub>, CClF<sub>3</sub>, C<sub>2</sub>F<sub>6</sub>, C<sub>2</sub>ClF<sub>5</sub>, CBrClF<sub>2</sub>, C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub> and C<sub>4</sub>F<sub>10</sub>, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

19. (Twice amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons [filled with] consisting of a physiologically acceptable gas [wherein the gas is] bounded by an organic polymer envelope at the gas/liquid interface, said

polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas;

and

substantially substituting at least a fraction of said first gas with a second gas which is the physiologically acceptable gas selected from the group consisting of SF<sub>6</sub>, CF<sub>4</sub>, CBrF<sub>3</sub>, C<sub>4</sub>F<sub>8</sub>, CClF<sub>3</sub>, C<sub>2</sub>F<sub>6</sub>, C<sub>2</sub>ClF<sub>5</sub>, CBrClF<sub>2</sub>, C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub> and C<sub>4</sub>F<sub>10</sub>, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

20. (Twice amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons [filled with] consisting of a gas mixture [wherein the gas mixture is] bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the steps of

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is the gas mixture comprising a physiologically acceptable gas selected from the group

consisting of SF<sub>6</sub>, CF<sub>4</sub>, CBrF<sub>3</sub>, C<sub>4</sub>F<sub>8</sub>, CClF<sub>3</sub>, C<sub>2</sub>F<sub>6</sub>, C<sub>2</sub>ClF<sub>5</sub>, CBrClF<sub>2</sub>, C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub> and C<sub>4</sub>F<sub>10</sub>, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

33. (Twice amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microballoons [being filled with] consisting of a physiologically acceptable gas [wherein the gas is] bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microballoons in the presence of the physiologically acceptable gas selected from the group consisting of SF<sub>6</sub>, CF<sub>4</sub>, CBrF<sub>3</sub>, C<sub>4</sub>F<sub>8</sub>, CClF<sub>3</sub>, C<sub>2</sub>F<sub>6</sub>, C<sub>2</sub>ClF<sub>5</sub>, CBrClF<sub>2</sub>, C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub> and C<sub>4</sub>F<sub>10</sub>, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference  $\Delta P$  between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25 Torr.

34. (Twice amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microballoons [being filled with] consisting of a gas mixture [wherein the gas mixture is]

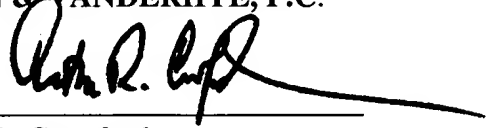
IV. Conclusion

For the reasons stated above, because pending claims 1-3, 6, 7, 13-22, 25, 26, and 30-48 are fully supported in the specification and are fully patentable over any references cited, favorable action on these claims is requested.

If there are any questions, the Examiner is respectfully asked to contact the Applicants' attorney.

Respectfully submitted,

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